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Selective molecular recognition of methylated lysines and arginines by cucurbit[6]uril and cucurbit[7]uril in aqueous solution†

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Cucurbit[7]uril selectively binds the epigenetic mark N^{ϵ},N^{ϵ},N^{ϵ}-trimethyllysine (LysMe$_3$, $K_{CB[7]}$ = (1.8 ± 0.6)× 10^6 dm^3 mol^{-1}) by 3500-fold over lysine ((5.3 ± 0.7) × 10^2 dm^3 mol^{-1}) in aqueous solution, using ion–dipole interactions and the hydrophobic effect, rather than cation–π interactions, as in the "aromatic cages" of *p*-SO$_3$-calix[4]arene hosts or chromodomain proteins which recognize LysMe$_3$. The trend in $K_{CB[7]}$ of LysMe$_3$ > LysMe$_2$ > LysMe > Lys follows the recognition pattern of the chromodomain HP1 and other LysMe$_n$ protein readers. With CB[6], protonation of the guest carboxylate group is required for the formation of inclusion complexes with the LysMe$_n$ series. The CB[7] host also displays modest selectivity between the asymmetric ((2.0 ± 0.3) × 10^3 dm^3 mol^{-1}) and symmetric ((6.1 ± 0.6) × 10^3 dm^3 mol^{-1}) dimethylarginines, both of which bind more strongly than the parent arginine or monomethylarginine.

Introduction

One mechanism of gene regulation is post-translation modifications (PTMs) of proteins, including histones, chromatin remodeling proteins, and transcriptional regulators.[1–3] The methylation of histone lysines (Fig. 1) plays a major role in transcriptional regulation and the abnormal methylation of lysines can lead to disease processes such as cancer.[4] The N^{ϵ}-trimethylation of lysines K4, K36, and K79 on histone H3 activate gene expression, while methylations of K9 and K27 inactive the process.[5] The methyllysine reader domains,[1] such as chromodomain HP1, use a protein "aromatic cage" comprised of two to four residues (tryptophan or tyrosine), and employing cation–π interactions with the polarized methyl groups, to induce new protein–protein interactions.[6] In addition, aspartate and glutamate residues can engage in direct or water-mediated H-bonding with NH protons on mono- and dimethyllysines. The plant homeodomains of the inhibitor of growth protein (ING2)[6c] and the human bromodomain/PHD domain transcription factor (BPTF),[7] and the HP1 chromodomain[8] are very selective for the different degrees of methylation of the histone H3 K4Me$_n$ residue, with binding constant ratios of about 1500 (ING2) and >1000 (HP1) for the K4Me$_3$ compared with the unmodified K4.[6c]

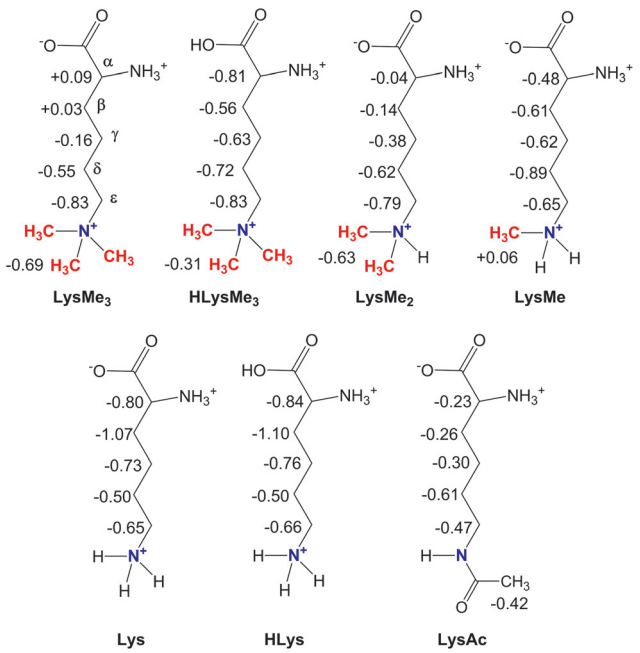

Fig. 1 The structures of the LysMe$_n$ and LysAc guest series. The values adjacent to the guest protons are the ^{1}H NMR chemical shift changes ($\Delta\delta_{lim}$, ppm) induced by cucurbit[7]uril complexation.

Recently, an unusual H3K4Me$_3$ recognition motif was discovered[9] in the cysteine-rich ADD$_{ATRX}$ domain associated with alpha-thalassemia and mental retardation X-linked syndrome. In addition to regular Glu H-bonding to the LysMe$_3$ backbone and cation–π interactions with one tyrosine, the remaining

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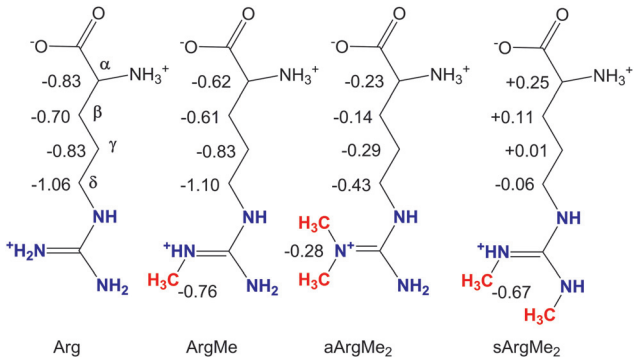

Fig. 2 Structures of arginine guests and their limiting complexation-induced ^{1}H NMR chemical shift changes ($\Delta\delta_{lim}$) upon forming a host–guest complex with cucurbit[7]uril in D_2O (pD = 4.7).

interactions were of the C–H···O type of hydrogen bonding[10] of the methyl protons with Tyr, Asp, Glu, and Ala residues.[9a] Interestingly, the protein–protein binding of ADD_{ATRX} is inhibited by increased methylation of the histone H3K9 residue.

Methylated arginines (Fig. 2) have been found in RNA-binding proteins and histones and recently have been linked to processes such as transcriptional regulation, signal transduction, RNA processing, and gametogenesis in the cell.[11] Asymmetric dimethylarginine ($aArgMe_2$) is an inhibitor of nitric oxide synthetases and elevated levels of this compound, as well as the symmetric dimethylarginine ($sArgMe_2$), which does not inhibit nitric oxide synthetase) are observed in patients with kidney disease and/or at risk for cardiovascular disease.[12] The $sArgMe_2$ has recently been determined as a biomarker for the acute kidney injury which commonly accompanies cardiac surgery. There is an interest in being able to distinguish between the two dimethylarginines in biological samples.[13] The protein modules known so far to recognize dimethylarginine modifications include the Tudor domains of survival motor neuron protein (SMN) and the survival of motor neuron-related splicing factor 30 (SPF30), which are important in the assembly of uridine-rich small nuclear ribonucleoproteins and in pre-mRNA splicing, respectively.[14] The binding of the WDR5 protein to histone H3 is observed to be enhanced through symmetric dimethylation of the R2Arg, while asymmetric dimethylation impedes the binding.[15]

The detection of PTMs in histone proteins is useful in an understanding of the gene regulatory histone code.[16] It is particularly desirable to have selective detection of tri-, di-, and monomethylated lysines (Fig. 1). Small host molecules with aromatic "walls" have recently been explored in terms of their selectivity in recognizing trimethyllysine *versus* lysine.[17] Waters and coworkers have used polyanionic carboxylated cyclophanes (*rac*-A_2B), identified through the use of dynamic combinatorial library studies, to bind to H3 histone tail peptides containing KMe_n residues and found that the binding constants and selectivities are similar to those of the natural HP1 peptide from chromodomain.[18] Hof and coworkers have used *p*-sulfonatocalix[4]arenes (CX4)[19] and derivatives[20,21] in which one of the SO_3^- groups is replaced with a variety of

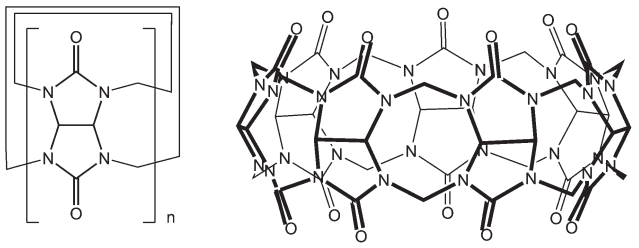

Fig. 3 Structures of the cucurbit[*n*]uril and CB[7] hosts.

substituents, to bind to the $LysMe_n$ guest series. Among the CX4 hosts, the parent molecule and the derivative with a phenyl replacing one SO_3^- group exhibit the strongest binding to $LysMe_3$ and the greatest binding selectivity relative to lysine. The Hof group has recently applied the library of CX4 derivatives to the development of a chemical sensor array for the antibody-free reading of methylated lysine and arginine residues on histone peptides.[22] Nau and co-workers have utilized CX4 with a fluorescent dye guest in a tandem enzyme assay[23,24] for monitoring lysine methyltransferase activity.[24] With the CX4 hosts,[19,20,24] the binding constants with $LysMe_3$ (up to 8×10^5 dm^3 mol^{-1}) and the $LysMe_3$–Lys ratio (up to 150) are also comparable with values observed with the HP1 histone on the chromodomain reader.[12] Neither the CX4[20] nor the $ArgMe_n$ protein readers[14] are particularly selective for the degree of methylation on arginine or in distinguishing between the symmetric and asymmetric isomers of dimethylarginine.

The cucurbit[*n*]uril (CB[*n*]) host molecules[25] have been compared with *p*-sulfonatocalixarenes in terms of their ability to bind cationic guests in aqueous solution.[23c,26] In this study, we have investigated the host–guest complexation of the $LysMe_n$ (n = 0–3) series (Fig. 1) by CB[6] and CB[7] and acetyllysine (Fig. 1) and the $ArgMe_n$ (n = 0, 1, and 2 (asymmetrical and symmetrical), Fig. 2) series of guests by CB[7] in aqueous media (Fig. 3). The CB[*n*] (n = 5–8, 10) hosts[25] are relatively rigid macrocycles comprised of n glycoluril units bridged by $2n$ methylene groups, whose complexations of guest molecules rely on ion–dipole, dipole–dipole, and hydrogen bonding interactions with the ureido carbonyl groups which rim the constrictive portals. In addition, the interior cavity provides for hydrophobic effects[27] and has a quadrupole moment which has been related to the alignment of encapsulated polar neutral guests.[27,28] The CB[7] host (Fig. 3) forms exceedingly stable host–guest complexes with cationic organic and organometallic guests ($K_{CB[7]}$ up to 10^{15} dm^3 mol^{-1},[29] rivalling the stability of the avidin–biotin complex) in aqueous solution, which has been exploited in applications such as the capturing of proteins labelled with ultra-high affinity guests by CB[7]-coated beads.[30] The complexation of acidic guests by CB[7] has a significant effects on their pK_a values, useful in the recognition and delivery of guests of biological and medicinal interest.[31]

The groups of Urbach[32] and Kim[33] have demonstrated that cucurbit[7]uril has a strong affinity for aromatic amino acid

residues such as phenylalanine (Phe) in peptides and proteins (1.5×10^6 dm^3 mol^{-1} for N-terminus Phe in insulin[34] and 2.8×10^6 dm^3 mol^{-1} in Phe–Gly–Gly[34]). They have also recently reported that CB[7] has a 20–30 fold higher affinity for non-canonical phenylalanine derivatives with either hydrophobic *t*-butyl or cationic ammoniomethyl groups at the *para* position, although only the latter substituent affords an increased affinity when part of a peptide chain.[35] Lysine, arginine, and histidine, which bear cationic side chains at physiological pH, exhibit much smaller binding constants to CB[7], in the range of 10^2–10^3 dm^3 mol^{-1}.[23,32]

We have observed that peralkylonium cations, including NR_4^+, can be bound (*e.g.* $K_{CB[7]}(NMe_4^+) = 1.2 \times 10^5$ dm^3 mol^{-1}) within the cavity of the CB[7] host molecule,[36] with selectivity based on R chain length. The trimethylammonium groups in these guests reside within the CB[7] cavity, as the delocalization of the positive charge over the methyl groups, renders this group relatively hydrophobic and conducive to CB[7] binding. The CB[7] molecule is also useful for complexations of other cationic guests possessing trimethylammonium centers, such as cholines[37] and acetylcholinesterase inhibitors,[38] in aqueous solution. This affinity may therefore be exploited for the selective molecular recognition of the epigenetic marker N^ε,N^ε,N^ε-trimethyllysine in the presence of lysine.

In this paper, the complexations of methylated and acetylated lysine and methylated arginine amino acids by CB[6] and CB[7] have been investigated using electrospray ionization mass spectrometry (ESI-MS) and ^{1}H NMR spectroscopy. The degree of selective molecular recognition has been determined through ^{1}H NMR titrations and competitive binding experiments and compared with results reported for other small molecule receptors and for native protein readers.

Experimental

Materials

Cucurbit[7]uril were prepared and characterized as described previously.[39] Cucurbit[6]uril (Fluka) and the N^ε,N^ε,N^ε-trimethyllysine hydrochloride (Sigma-Aldrich), N^ε,N^ε-dimethyllysine hydrochloride (Bachem), N^ε-methyllysine hydrochloride (Bachem), L-lysine (Sigma-Aldrich), acetyllysine (Sigma-Aldrich), L-arginine hydrochloride (Sigma-Aldrich), *N*-methylarginine acetate (Sigma-Aldrich), and asymmetric dimethylarginine hydrochloride (Sigma-Aldrich) guests were used as received. The *p*-hydroxyazobenzene-*p*′-sulfonate salt of the symmetric dimethylarginine (Sigma-Aldrich) was converted to the chloride salt by ion-exchange using Amberlite IRA-400 anion exchange resin (Fisher).

Methods

The ^{1}H NMR spectra and chemical shift titrations were recorded on Bruker Avance AM 400 and 500 MHz instruments. The electrospray ionization mass spectrometry (ESI-MS) experiments were carried out on a QStar XL TOF instrument. The stability constants for the host–guest complexes formed between cucurbit[7]uril and $LysMe_3$, $LysMe_2$, and Lys were determined by means of competitive ^{1}H NMR binding experiments. The protocol of Isaacs and coworkers[40] was employed and the measurements were taken at 25 °C in D_2O containing an acetate buffer (50 mmol dm^{-3} NaO_2CCD_3/0.25 mmol dm^{-3} DCl) at pD = 4.7. The competitor guests employed were $(CH_3)_3Si(CD_2)_2COOH$ (for $LysMe_3$): $K_{CB[7]} = (1.82 \pm 0.22) \times 10^5$ dm^3 mol^{-1},[40] $N(CH_2CH_3)_4Br$ (for $LysMe_3$): $K_{CB[7]} = (1.0 \pm 0.2) \times 10^6$ dm^3 mol^{-1},[36] $N(CH_3)_4I$ (for protonated Lys and $LysMe_2$): $K_{CB[7]} = (1.2 \pm 0.4) \times 10^5$ dm^3 mol^{-1},[36] $P(CH_3)_4Br$ (for protonated Lys and $LysMe_3$): $(2.2 \pm 0.4) \times 10^6$ dm^3 mol^{-1},[36] and acetone (for Lys): $(4.5 \pm 0.5) \times 10^2$ dm^3 mol^{-1} (interpolated from the values at $[Na^+] = 0.00$ (580 dm^3 mol^{-1}) and 0.20 mol dm^{-3} (370 dm^3 mol^{-1}) in ref. 28). The stability constants for the host–guest complexes formed between CB[7] and LysMe, $sArgMe_2$ and $aArgMe_2$ were determined by means of ^{1}H NMR chemical shift titrations of the guests with CB[7] at 25 °C in D_2O containing an acetate buffer (50 mmol dm^{-3} NaO_2CCD_3/0.25 mmol dm^{-3} DCl) at pD = 4.7). The ^{1}H NMR titrations of the $LysMe_n$ guests with CB[6] were carried out in D_2O containing 0.10 mol dm^{-1} NaCl with or without 0.10 mol dm^{-3} DCl (pD = 1.0). The complexation-induced chemical shift changes for the proton resonances, as a function of [CB[*n*]], were fit to a 1 : 1 binding model as described previously.[41] For the weakest host–guest binding, Benesi–Hildebrand plots[42] of $\Delta\delta_{obs}^{-1}$ *versus* $[CB[n]]^{-1}$ were constructed and the values of $\Delta\delta_{lim}$ and $K_{CB[n]}$ were calculated from the reciprocal of the intercept and the ratio of the slope/intercept, respectively.

Results and discussion

Methylated lysine guests

The formation of 1 : 1 host–guest complexes between CB[7] and the $LysMe_n$, guests were confirmed by electrospray ionization mass spectrometry (ESI-MS) and by ^{1}H NMR spectroscopy. The mass spectra exhibited peaks for both $\{LysMe_n{\cdot}CB[7]\}^+$ and $\{LysMe_n{\cdot}CB[7]{\cdot}H\}^{2+}$ (Table 1), as a result of the proton equilibrium of the guest carboxylic acid group in the gas phase. The interior cavity of CB[7] provides a shielding environment for guest protons, with upfield complexation-induced ^{1}H NMR chemical shift changes ($\Delta\delta_{lim} = \delta_{bound} - \delta_{free}$) inferring guest proton encapsulation, while downfield shifts are observed for guest protons outside of the cavity adjacent to the polar portals. The complexation of $LysMe_3$ results in upfield shifts in methyl and H_γ–H_ε proton resonances (Fig. 1), while the H_α and H_β protons resonances shift slightly downfield (see ESI,† Fig. S1). The limiting value of $\Delta\delta$ for the methyl protons on $LysMe_3$ (−0.69 ppm) is similar to those of the $N(CH_3)_4^+$ cation (−0.72 ppm)[36] and choline (−0.66 ppm)[37] bound to CB[7], suggesting that the NMe_3^+ group is located within the cavity of the host.

With the reduction in the number of methyl groups on the quaternary N_ε center and hence its hydrophobicity, there is a translocation of the average location of the CB[7] host from the N_ε end of the guest towards N_α end, reflected in the relative

magnitudes of the chemical shift changes of the H_β–H_ε methylene proton resonances (Fig. 1, and see ESI,† Fig. S1–S3 and S5). With the $LysMe_2$ and LysMe guests, the maximum upfield $\Delta\delta$ value is observed for the H_ε and H_δ protons, respectively, while for lysine, the H_β proton exhibits the largest upfield shift. The titration spectra for $LysMe_n$ (n = 1–3) exhibit intermediate to fast guest exchange behaviour with broadening of the guest resonances located within the cavity of the host. In the case of lysine, there is considerable broadening of the guest proton resonances, suggesting a slower rate of dissociation from the host cavity. This is likely due to greater difficulty in the passage of the hydrophilic ammonium group through the hydrophobic cavity than was the case in the methylated derivatives.

Table 1 ESI-MS data for the $\{LysMe_n{\cdot}CB[7]\}^+$ and $\{ArgMe_n{\cdot}CB[7]\}^+$ complexes in aqueous solution

Complex (M)	m/z ($M + H^{2+}$)	m/z (M^+)
$\{Lys{\cdot}CB[7]\}^+$	obs. 655.2314 calc. 655.2318 for $C_{48}H_{58}N_{30}O_{16}^{2+}$	
$\{LysMe{\cdot}CB[7]\}^+$	obs. 662.2239 calc. 662.2397 for $C_{49}H_{60}N_{30}O_{16}^{2+}$	obs. 1323.4228 calc. 1323.4720 for $C_{49}H_{59}N_{30}O_{16}^{+}$
$\{LysMe_2{\cdot}CB[7]\}^+$	obs. 669.2384 calc. 669.2475 for $C_{50}H_{62}N_{30}O_{16}^{2+}$	obs. 1337.4939 calc. 1337.4877 for $C_{50}H_{61}N_{30}O_{16}^{+}$
$\{LysMe_3{\cdot}CB[7]\}^+$	obs. 676.2475 calc. 676.2553 for $C_{51}H_{64}N_{30}O_{16}^{2+}$	obs. 1351.5029 calc. 1351.5033 for $C_{51}H_{63}N_{30}O_{16}^{+}$
$\{AcLys{\cdot}CB[7]\}^+$		obs. 1351.4651 calc. 1351.4674 for $C_{50}H_{59}N_{30}O_{18}^{+}$
$\{Arg{\cdot}CB[7]\}^+$	obs. 669.2357 calc. 669.2348 for $C_{48}H_{58}N_{32}O_{16}^{2+}$	
$\{ArgMe{\cdot}CB[7]\}^+$	obs. 676.2440 calc. 676.2427 for $C_{49}H_{60}N_{32}O_{16}^{2+}$	
$\{aArgMe_2{\cdot}CB[7]\}^+$		obs. 1365.4918 calc. 1365.4943 for $C_{50}H_{61}N_{32}O_{16}^{+}$
$\{sArgMe_2{\cdot}CB[7]\}^+$		obs. 1365.4969 calc. 1365.4943 for $C_{50}H_{61}N_{32}O_{16}^{+}$

The stability constants for the host–guest complexes formed between cucurbit[7]uril and the $LysMe_n$ guest series (Table 2, in D_2O, with 50 mmol dm^{-3} NaOAc buffer, pD = 4.7) were determined by means of ^1H NMR competitive binding experiments,[40] with the exception of the value for LysMe (1.8 ± 0.2) $\times 10^3$ dm^3 mol^{-1}) which was calculated from a complexation-induced ^1H NMR chemical shift titration (see ESI,† Fig. S5). For $LysMe_3$, the values of (2.3 ± 0.3) $\times 10^6$ dm^3 mol^{-1} (determined using 3-trimethylsilylpropionic acid)[40] and (1.3 ± 0.2) $\times 10^6$ dm^3 mol^{-1} (using NEt_4^+)[34] are in good agreement. This binding constant may be compared with that for choline by CB[7], $K_{CB[7]}$ = (6.5 ± 1.2) $\times 10^5$ dm^3 mol^{-1}, in which the trimethylammonium group is also encapsulated in the inner cavity ($\Delta\delta_{lim}$(Me) = −0.66 ppm).[37] The CB[7] binding constants for $LysMe_2$ (using NMe_4^+)[36] and Lys (using acetone)[28] were determined to be (6.0 ± 2.5) $\times 10^4$ and (5.3 ± 0.7) $\times 10^2$ dm^3 mol^{-1}, respectively. The value for lysine is lower that with the binding constant reported by Nau and coworkers[23b] (Table 2), as a higher buffer (cation) concentration was used in the present study compared with Nau's measurement (10 mmol dm^{-3} NH_4OAc).

The pK_a of the protonated lysine is 2.2 and a titration of lysine at pD = 2.0 (0.010 mol dm^{-3} DCl and 0.050 mol dm^{-3} NaCl, see ESI,† Fig. S6) revealed similar values of $\Delta\delta_{lim}$ to those observed at pD = 4.7 (Fig. 1). The protonated lysine has positive charges at either end of the guest, and the positioning of these charges near the two portals would place the H_α–H_ε protons with the cavity of CB[7], accounting for the significant upfield shifts in all of the resonances. The binding constant

Table 2 Binding constants (dm^3 mol^{-1}) for free $LysMe_n$ amino acid guests with synthetic receptors (CB[7], CX4 and CX4*) and histone H3 $LysMe_n$ with synthetic (*rac*-A_2B) and protein receptors (HP1, ING2, ADD_{ATRX}, and BPTF), and the selectivity ratio of the binding constants for $LysMe_3$/Lys

Host	K ($LysMe_3$)	K ($LysMe_2$)	K (LysMe)	K (Lys)	Selectivity $LysMe_3$/Lys
CB[7]	$1.9 \times 10^{6a,b}$	6.0×10^{4a}	1.8×10^{3a}	5.3×10^{2a} 8.7×10^{2c}	3500
CX4	3.7×10^{4d} 1.3×10^{5e}	1.6×10^{4d} 6.0×10^{4e}	4.0×10^{3d} 2.0×10^{4e}	5.2×10^{2d} $<1 \times 10^{3e}$	70 >130
CX4*[f]	6.4×10^{4f}	nd	nd	4.2×10^{2f}	150
rac-A_2B	4.0×10^{4g}	1.7×10^{4g}	6.0×10^{3g}	$<8 \times 10^{2g}$	>50
HP1	1.0×10^{5h} 4.8×10^{5i}	6.7×10^{4h} 2.6×10^{4i}	1.0×10^{4h}	$<1 \times 10^{3h}$	>100
ING2	6.7×10^{5j}	6.7×10^{4j}	4.8×10^{3j}	4.5×10^{2j}	1500
ADD_{ATRX}	2.0×10^{6k} 3.6×10^{6l} 7.7×10^{5m}	7.7×10^{5k} 2.6×10^{6l}	4.0×10^{5k} 3.4×10^{5l} 1.2×10^{5m}	2.7×10^{5k} 1.3×10^{5l} $<1 \times 10^{4m}$	7.4 28 >70
BPTF	3.7×10^{5n}	2.0×10^{5n}			

[a] This work, 50 mmol dm^{-3} NaOAc buffer, pD 4.7. [b] Average of two measurements using different competitors. [c] Ref. 23*b*, 10 mmol dm^{-3} NH_4OAc buffer, pH 6. [d] Ref. 19, 40 mmol dm^{-3} phosphate buffer, pH 7.4. [e] Ref. 23, 5 mmol dm^{-3} glycine buffer, pH 10. [f] Ref. 20, CX4 with one SO_3^- group replaced by phenyl, 40 mmol dm^{-3} phosphate buffer, pH 7.4. [g] Ref. 17, 10 mmol dm^{-3} phosphate buffer, pH 8.5, 27 °C. [h] Ref. 8*a*, phosphate buffer, pH 7.5, 15 °C. [i] Ref. 12, 50 mmol dm^{-3} phosphate buffer, 25 mmol dm^{-3}, pH 8.0, 25 °C. [j] Ref. 6*c*. [k] $H3_{1-15}$, ref. 9*a*, 100 mmol dm^{-3} KCl, pH 7.5. [l] $H3_{1-15}$, ref. 9*b*, 100 mmol dm^{-3} NaCl, 50 mmol dm^{-3} Tris, pH 7.0. [m] $H3_{1-19}$, ref. 9*c*, 100 mmol dm^{-3} KCl, 25 mmol dm^{-3} Tris, pH 8.0. [n] Ref. 7.

for the protonated lysine with CB[7] was determined to be (8.8 ± 0.9) × 10^5 and (1.7 ± 0.2) × 10^6 dm^3 mol^{-1} from competitive ^1H NMR binding experiments using the $N(CH_3)_4^+$ and $P(CH_3)_4^+$ cations,[36] respectively. The binding constant for the protonated lysine is comparable to the value of 1.4 × 10^7 dm^3 mol^{-1} (10 mmol dm^{-3} NH_4OAc, pH 6.0) for CB[7] binding of its decarboxylation product, cadaverine (1,5-diammoniopentane dication).[23b]

A ^1H NMR titration of $LysMe_3$ at pD = 2.0 (0.010 mol dm^{-3} DCl and 0.050 mol dm^{-3} NaCl, see ESI,† Fig. S7) indicated that the CB[7] is located more over the center of the protonated guest than observed for its conjugate base (Fig. 1). The trimethylammonium group is closer to a portal, allowing the ammonium group on the other end of the guest to lie adjacent to the other portal. The binding constant for the protonated trimethyllysine with CB[7] was determined to be (2.0 ± 0.3) × 10^7 dm^3 mol^{-1} from competitive ^1H NMR binding experiments using the $P(CH_3)_4^+$ cation.[36]

The increase in the binding constant for the protonated $LysMe_3$ compared with its conjugate base is only one order of magnitude, while a 6000-fold increase is observed with lysine. For lysine, the increase can be related to the neutralization of the negative charge on the carboxylate group, as the NMR data suggest that the position of CB[7] does not change upon protonation. With $LysMe_3$, the smaller change in binding constant may represent compensating effects of an increase in the ion–dipole interaction upon protonate and a decrease in the hydrophobic effect upon movement of the trimethylammonium group away from the center of the inner cavity of CB[7], as suggested by the NMR chemical shift changes.

Complexations of methylated lysines by CB[6]

While stability constant for the binding of lysine to the smaller CB[6] host in aqueous solution has not been reported, the lysine residue in the Lys–Ala–NH_2 dipeptide forms a more stable complex with CB[6] (K = 1.6 × 10^4 dm^3 mol^{-1} in 0.10 mol dm^{-3} NaCl solution)[33] than does Lys with CB[7] in solution. In the same study, the ^1H NMR spectrum of a mixture of the Lys–Tyr and Tyr–Lys dipeptides with CB[6] and CB[7] indicated that self-sorting resulted in the formations of {CB[6]·Lys–Tyr} and {CB[7]·Tyr–Lys} host–guest complexes. In the gas phase the complexation between CB[6] and the protonated lysine residues, as detected by electrospray MS,[43] has been used to determine the structures of proteins through collisionally activated experiments.[44] Titrations of the $LysMe_n$ guests with CB[6] in D_2O containing 0.10 mol dm^{-3} NaCl, in the present work (see ESI,† Fig. S8–S11), indicate that the monocationic species form only exclusion complexes at a portal, with $K_{CB[6]}$ = 1.1 × 10^4 dm^3 mol^{-1} for lysine (see ESI,† Fig. S12). Similar behaviour has been observed for other amino acids with hydrophobic side chains, such as glycine and alanine ($K_{CB[6]}$~ 10^3 dm^3 mol^{-1}).[45] When the pD is reduced to 1.0 (0.10 mol dm^{-3} DCl, 0.10 mol dm^{-3} NaCl), however, inclusion complexes are observed with lysine and the methylated derivatives (see ESI,† Fig. S13–S16), consistent with the gas-phase studies which detected CB[6] complexes with only the protonated form of lysine. The protonated lysine exhibits large upfield chemical shift changes (see ESI,† Fig. S17) with slow exchange behaviour and the binding constant is calculated to be 1400 ± 300 dm^3 mol^{-1}. This value is than that of 1,5-cadaverine with CB[6] in 50% aqueous formic acid, 2.4 × 10^6 dm^3 mol^{-1},[46] perhaps due to the presence of the Na^+. The protonated HLysMe, $HLysMe_2$, and $HLysMe_3$ guests exhibit fast exchange behaviour, with stability constants of 990 ± 170, 270 ± 60, and 1130 ± 230 dm^3 mol^{-1}, respectively (see ESI,† Fig. S17). The methyl proton resonance of HLysMe shifts downfield, while those of $HLysMe_2$ and $HLysMe_3$ exhibit upfield shifts upon CB[6] complexation (see ESI,† Fig. S18).

Kim and co-workers[47] have reported that choline (Me_3N-$(CH_2)_2OH^+$) does not bind to hexa(cyclohexyl)-cucurbit[6]uril, although this host does form a complex with the acetylcholine guest (K = 1.3 × 10^3 dm^3 mol^{-1}), with the trimethylammonium group outside of the cavity. The CB[6]-induced upfield shifts in the methyl resonances of $HLysMe_3$ and $HLysMe_2$ indicate that the quaternary ammonium group are located within the cavity in these host–guest complexes.

Acetylated lysine guest

In addition to the studies of the methylated lysines, the host–guest binding constant for CB[7] with the neutral acetyllysine guest was determined from a ^1H NMR chemical shift titration in D_2O (with 50 mmol dm^{-3} NaOAc buffer, pD = 4.7, see ESI,† Fig. S19). All of the guest proton resonances shifted upfield (Fig. 1) and a Benesi–Hildebrand plot of the methyl proton chemical shift change (see ESI,† Fig. S20) yielded a stability constant of 20 ± 10 dm^3 mol^{-1}. With the *p*-sulfonatocalix[4]-arene, a small binding constant for acetyllysine of 12 ± 34 dm^3 mol^{-1} has been reported.[19] The bromodomain protein readers of post-translational acetyllysine utilize a hydrophobic cavity with hydrogen bonding to a conserved asparagine residue.[48] The lack of the quaternary ammonium center found in lysine and its methylated derivatives (and the resulting ion–dipole interactions with the portal carbonyl groups) accounts for the decrease in the binding with respect to the other lysine guests.

Methylated arginine guests

The formation of 1 : 1 host–guest complexes of the $ArgMe_n$ series of guests in aqueous solution is indicated by the ESI mass spectral data (Table 1) and the ^1H NMR spectra (ESI,† Fig. S21–S24). The trend in the values of $\Delta\delta_{lim}$ for the arginine guests is similar to the lysine series in terms of the implied shift in the position of the CB[7] host as methyl groups are added to the guanidinium moiety (Fig. 3). The two dimethylarginines appear to bind to CB[7] in somewhat different fashions, with the CB[7] including most of the a$ArgMe_2$ guest within its inner cavity. With the s$ArgMe_2$ guest, it is clear from the ^1H NMR chemical shift changes that the dimethylguanidinium group is encapsulated, while the remaining portion of the amino acid is within the portal or outside of the host.

Both the parent L-arginine and the ArgMe guest displayed weak binding to the CB[7] host (Table 3), with stability constants of $K_{CB[7]}$ = 190 ± 50 (see ESI,† Fig. S25) and 110 ± 30 dm^3 mol^{-1}

Table 3 Binding constants (dm^3 mol^{-1}) for free $ArgMe_n$ with synthetic receptors (CB[7] and CX4) and histone $R4ArgMe_n$ (with SND1 and SMN proteins) and $R2ArgMe_n$ (with WDR5 protein) with protein receptors, and the selectivity ratio of the binding constants for $sArgMe_2$/$aArgMe_2$

Host	K ($sArgMe_2$)	K ($aArgMe_2$)	K (ArgMe)	K (Arg)	Selectivity $sArgMe_2$/$aArgMe_2$
CB[7]	6.1×10^{3a}	2.0×10^{3a}	1.1×10^{2a}	1.9×10^{2a} 3.1×10^{2b}	3
CX4	1.1×10^{3c}	1.1×10^{3c} 1.3×10^{3d}	7.6×10^{2c}	3.3×10^{2c}	1
SND1	1.0×10^{5e}	2.4×10^{4e}	5.3×10^{4e}	1.1×10^{4e}	4
SMN	2.9×10^{4f}	1.0×10^{4f}	$<6.7 \times 10^{3f}$	$<3.3 \times 10^{3f}$	3
WDR5	1.0×10^{7g}			1.8×10^{5g}	

[a] This work, 50 mmol dm^{-3} NaOAc buffer, pD 4.7. [b] Ref. 23*b*, 10 mmol dm^{-3} NH_4OAc buffer, pH 6. [c] Ref. 20, 40 mmol dm^{-3} phosphate buffer, pH 7.4. [d] ITC measurement. [e] Ref. 14*a*, 50 mmol NaCl, pH 7.5. [f] Ref. 14*d*, 50 mmol dm^{-3} NaCl, pH 7.5. [g] Ref. 15*c*.

(see ESI,† Fig. S26), respectively. A value of 310 dm^3 mol^{-1} for L-arginine with CB[7] has been reported by Nau and coworkers[23b] in a pH 6.0 medium containing 10 mmol dm^{-3} NH_4OAc, with the lower cation concentration accounting for the larger value. The dimethylarginine guests exhibits stronger binding interactions with CB[7], and fits of the chemical shift changes of the $aArgMe_2$ Hδ and $sArgMe_2$ methyl proton resonances to a 1 : 1 binding model (see ESI,† Fig. S27–S28) yielded values of $K_{CB[7]} = (2.0 \pm 0.3) \times 10^3$ and $(6.1 \pm 0.9) \times 10^3$ dm^3 mol^{-1}, respectively.

Comparisons with other hosts

Tables 2 and 3 also provide the corresponding values for the complexations of the free $LysMe_n$ guests with the *p*-sulfonatocalix[4]arenes,[19,20,24] as well as for the binding of K4 $LysMe_n$ residues by a polyanionic cyclophane host (*rac*-A_2B),[18] the HP1 histone of chromodomain[8] and the inhibitor of plant growth, ING2.[6c] The binding constant for $LysMe_3$ with CB[7] is the largest so far reported for small molecule receptors, and the binding is achieved without a reliance on ion–ion or cation–π interactions. The binding relies instead on the ion–dipole interactions and the hydrophobic effect, with the decrease in the CB[7] stability constants on going from $LysMe_3$ to Lys (see ESI,† Fig. S29) being related to the decreasing hydrophobicity of the terminal ammonium group as methyl groups are replaced by protons. Fig. 4 shows a titration of a mixture of Lys and $LysMe_3$ with CB[7]. As expected from the 3500-fold selectivity for $LysMe_3$ over Lys, the complexation of the $LysMe_3$ is complete (Fig. 4(d), at about one equiv. CB[7]) before there is any change in the proton resonances of the Lys guest.

The host–guest stability constants for the binding of Arg, ArgMe, and asymmetrical $ArgMe_2$ are comparable to those reported for the *p*-sulfonatocalix[4]arene by Hof and coworkers.[20] The CB[7] has somewhat less selectivity between the ArgMe and the parent Arg guest, but more affinity towards the asymmetric $ArgMe_2$ than ArgMe or Arg. The CB[7], however, shows modest selectivity for the symmetric $ArgMe_2$ over the asymmetric $ArgMe_2$, by a factor of three, while the CX4 host has little if any selectivity. Small selectivity in favour of the symmetric $ArgMe_2$ has recently been observed for recognition on the Tudor domain survival motor neuron protein (SMN) and the SND1 (Table 3).[14] These methylarginine readers bind the series of $ArgMe_n$ residues with stability constants in the range of 10^3–10^7 dm^3 mol^{-1}, with a trend towards higher binding with increased methylation. The 30-fold selectivity exhibited by CB[7] for free $sArgMe_2$ over Arg is only slightly lower than the 50-fold selectivity shown by the WDR5 protein for $sArgMe_2$ and Arg residues on the H3 tail.[15c]

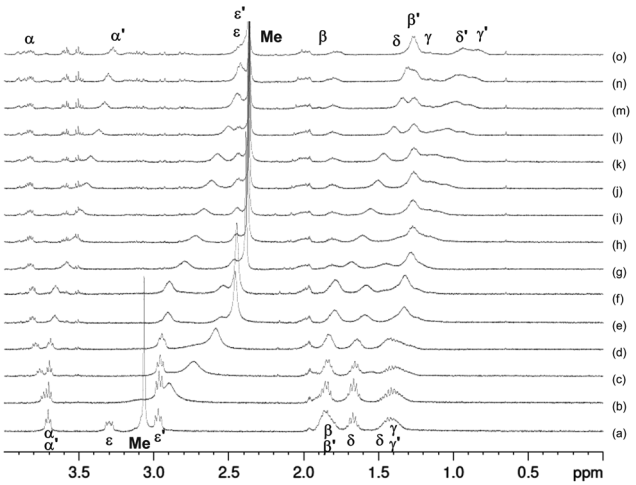

Fig. 4 ^1H NMR titration of a mixture of $LysMe_3$ (0.85 mmol dm^{-3}) and Lys (1.00 mmol dm^{-3}, labelled prime symbols) with CB[7] in D_2O (50 mmol dm^{-3} NaOAc, pD = 4.7): (a) 0.00, (b) 0.32, (c) 0.62, (d) 0.96, (e) 1.40, (f) 1.62, (g) 1.92, (h) 2.64, (i) 2.81, (j) 3.20, (k) 3.39, (l) 3.52, (m) 4.24, (n) 4.70, and (o) 6.01 mmol dm^{-3} CB[7].

While the present study indicates that CB[7] exhibits considerable selectivity in binding the noncanonical amino acid $LysMe_3$ over its natural counterpart and modest recognition of the symmetrical $ArgMe_2$ over its asymmetrical isomer, this may or may not translate to similar recognition in peptides or proteins, in which the respective amino acid residues may be on a buried sidechain or adjacent to different neighbouring surface residues.[1,32] The observation that CB[7] prefers to bind, based on the magnitudes of the complexation-induced chemical shift changes, to $LysMe_3$ (Fig. 1) and $sArgMe_2$ (Fig. 2) over the trimethylammonium and dimethylguanidinium end groups, respectively, rather than closer to aminocarboxylate end of the guests, as with lysine and $aArgMe_2$, would suggest that the binding selectivity (if not the binding strengths)

observed with the free amino acids might be further enhanced in such residues on a peptide chain. Further investigations into the applications of CB[7] for sensing $LysMe_3$ in post-translationally modified peptides and proteins are needed.

Conclusions

In conclusion, the neutral, non-aromatic host molecule cucurbit[7]uril exhibits the largest binding constants, to date, for small molecule receptors of the noncanonical N^{ε},N^{ε},N^{ε}-trimethyllysine and symmetrical dimethylarginine, as well as the greatest degree of selectivity for the trimethyllysine over lysine, and modest selectivity of symmetrical over asymmetrical dimethylarginine. The incremental increase in the binding constant upon methylation of the guests may be related to hydrophobic effects and ion–dipole interactions between the $LysMe_n$ and $ArgMe_n$ guests and the CB[7] host.

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